CLAIMS

What is claimed is:

1. A reporting system for monitoring real-time gene expression events in a cell, comprising:

an illuminogenic substrate, wherein said substrate is contained within said cell; and

at least one reporter protein, wherein said reporter protein facilitates the conversion of said illuminogenic substrate into an illuminescent molecule, and wherein said reporter protein has a short cellular life time.

2. The system of claim 1, wherein said cell can be either a prokaryotic cell or an euckaryotic cell.

3. The system of claim 1, wherein said illuminogenic substrate is a fluorogenic substrate.

4. The system of claim 3, wherein said fluorogenic substrate is selected from the group consisting of DDAO-galactopyranoside, resorufin-galactopyranoside, resorufin-glucopyranoside, DDAO-glucopyranoside, CCF2, CR2 and alike.

5. The system of claim 1, wherein said system has high sensitivity for low copy number proteins.

6. The system of claim 1, wherein said reporter protein comprises enzymatic activity.

7. The system of claim 6, wherein said enzymatic activity is selected from the group consisting of β-galactosidase, β-glucosidase, β-lactamase, and alike.

8. The system of claim 1, wherein said short cellular life time for said reporter protein ranges from about 2 minutes to about half an hour.

9. The system of claim 1, wherein said reporter protein is constructed of an amino acid sequence using the N-end rule in order to shorten the cellular life time of said reporter protein.

10. The system of claim 9, wherein a methionine residue positioned at the amino terminus of said reporter protein is replaced with an amino acid selected from the group consisting of leucine, arginine, lysine, phenylalanine, tryptophan, and tyrosine.

11. The system of claim 10, wherein an ubiquitin nucleotide fusion construct is used to introduce a predetermined nucleotide sequence that encodes for a predetermined amino acid sequence that is expressed resulting in said reporter protein.

12. The system of claim 11, wherein an ubiquitin fusion expression peptide product is selected from the group consisting of Ub-leu-β-gal, Ub-arg-β-gal, and alike.

13. The system of claim 12, wherein said ubiquitin peptide fusion construct is Ub-arg-*lacZ*.

14. The system of claim 13, wherein a nucleotide sequence for said Ub-arg-*lacZ* is SEQ ID NO. 1, and wherein an amino acid sequence for said Ub-arg-*lacZ* is SEQ ID NO. 2.

15. The system of claim 12, wherein said ubiquitin fusion peptide product is Ub-arg-β-gal.

16. The system of claim 15, wherein said Ub-arg-β-gal has a linker sequence between ubiquitin and β-gal.

17. The system of claim 16, wherein said linker sequence comprises an amino acid sequence selected from group consisting of SEQ ID NO. 9, SEQ ID NO. 11, and SEQ ID NO. 13.

18. The system of claim 17, wherein said linker sequence is encoded by a nucleotide nucleotide sequence selected from the group consisting of SEQ ID NO. 10, SEQ ID NO. 12, and SEQ ID NO. 14.

19. The system of claim 1, wherein said reporter protein comprise a N-terminal signal amino acid sequence operatively linked to a β-gal amino acid sequence in order to shorten the cellular life time of said reporter protein, wherein said N-terminal signal amino acid sequence is selected from the group consisting of SEQ ID NO. 15 and SEQ ID NO. 17.

20. The system of claim 19, wherein said N-terminal signal amino acid sequence is encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO. 16 and SEQ ID NO. 18.

21. The system of claim 1, wherein a nucleotide sequence encoding for said reporter protein is operatively linked within the genome of said cell.

22. The system of claim 21, wherein said nucleotide sequence encoding for said reporter protein is operatively linked to a host cell nucleotide sequence that encodes a host specific protein.

23. A live-cell microarray, comprising multiple libraries of cells each of which differs from the rest in at least one genotypic property.

24. The microarray of claim 23, wherein said cells are selected from the group consisting of prokaryotic cells and eukaryotic cells.

25. The microarray of claim 23 comprising two libraries of cells.

26. The microarray of claim 25, wherein a first library of cells has cells that have a promoterless *lacZ* gene that encodes for a short lived β-gal having its own ribosome binding site, wherein said promoterless *lacZ* gene is inserted within a host's promoter region, and wherein a second library of cells has the same elements as said first library of cells except that a gene encoding for a short lived YFP replaces said gene encoding for said short lived β-gal.

27. The microarray of claim 26, wherein said YFP is a Venus-ssrA nucleotide construct.

28. The microarray of claim 27, wherein said Venus-ssrA nucleotide construct encodes a ssrA peptide tag, wherein said ssrA peptide tag amino acid sequence is SEQ ID NO. 21.

29. The microarray of claim 28, wherein said Venus-ssrA nucleotide construct is operatively linked to a nucleotide sequence within the pVS5 plasmid, and wherein said Venus-ssrA nucleotide sequence is SEQ ID NO. 22, wherein said ssrA nucleotide encodes for an amino acid sequence, and wherein said amino acid sequence is SEQ ID NO. 23.

30. A method of monitoring gene expression in real-time in a living cell, comprising the steps of:

(a) obtaining a cell population in which at least one cell comprises at least one nucleotide sequence encoding a reporter protein operatively linked to said cell's genome, wherein said reporter protein when expressed has enzymatic activity and has a short cellular life time;

(b) introducing an illuminogenic substrate to said cell under conditions suitable for the conversion of said illuminogenic substrate to an illuminescent molecule, wherein said substrate enters said cell; and

(c) detecting an illuminescent signal.

31. The method of claim 30, wherein the cell population can comprise either prokaryotic cells, eukaryotic cells, or a combination of both.

32. The method of claim 31, wherein the cell population is selected from the group consisting of prokaryotic cells and eukaryotic cells.

33. The method of claim 30, wherein said nucleotide sequence encoding for a reporter protein is operatively linked to a predetermined host cell's nucleotide sequence that encodes a specific protein.

34. The method of claim 30, wherein said reporter protein comprises an amino acid sequence selected from the group consisting of Ub-leu-β-gal, Ub-arg-β-gal, and alike.

35. The method of claim 30, wherein said illuminogenic substrate is a fluorogenic substrate.

36. The method of claim 35, wherein said fluorogenic substrate is selected from the group consisting of DDAO-galactopyranoside, resorufin-galactopyranoside, resorufin-glucopyranoside, DDAO-glucopyranoside, CCF2, CR2 and alike.

37. The method of claim 30, wherein said enzyme activity is selected from the group consisting of β-galactosidase, β-glucosidase, β-lactamase, and alike.

38. The method of claim 30, wherein said reporter protein comprises a substituted amino acid in place of the amino-terminus methionine residue.

39. The method of claim 38, wherein said substituted amino acid is selected from the group consisting of leucine, arginine, lysine, phenylalanine, tryptophan, and tyrosine.

40. The method of claim 30, wherein said detection is accomplished by any means of detecting a signal including visible and UV spectrometry, fluorometry, and alike.

41. A method for cell sorting, comprising the steps of:

(a) obtaining a cell population in which at least one cell comprises at least one nucleotide sequence encoding a reporter protein operatively linked to said cell's genome, wherein said reporter protein when expressed has enzymatic activity and has a short cellular life time;

(b) introducing an illuminogenic substrate to said cell under conditions suitable for the conversion of said illuminogenic substrate to an illuminescent molecule, wherein said substrate enters said cell;

(c) contacting said cell population with a perturbing agent; and

(d) detecting an illuminescent signal.

41. The method of claim 40, wherein the cell population can comprise either prokaryotic cells, eukaryotic cells, or a combination of both.

42. The method of claim 41, wherein the cell population is selected from the group consisting of prokaryotic cells and eukaryotic cells.

43. The method of claim 40, wherein said nucleotide sequence encoding for a reporter protein is operatively linked to a predetermined host cell's nucleotide sequence that encodes a specific protein.

44. The method of claim 40, wherein said reporter protein comprises an amino acid sequence selected from the group consisting of Ub-leu-β-gal, Ub-arg-β-gal and alike.

45. The method of claim 40, wherein said illuminogenic substrate is a fluorogenic substrate.

46. The method of claim 45, wherein said fluorogenic substrate is selected from the group consisting of DDAO-galactopyranoside, resorufin-galactopyranoside, resorufin-glucopyranoside, DDAO-glucopyranoside, CCF2, CR2 and alike.

47. The method of claim 40, wherein said enzyme activity is selected from the group consisting of β-galactosidase, β-glucosidase, β-lactamase, and alike.

48. The method of claim 40, wherein said reporter protein comprises a substituted amino acid in place of the amino-terminus methionine residue.

49. The method of claim 48, wherein said substituted amino acid is selected from the group consisting of leucine, arginine, lysine, phenylalanine, tryptophan, and tyrosine.

50. The method of claim 40, wherein said detection is accomplished by any means of detecting a signal including visible and UV spectrometry, fluorometry, and alike.